Mechanism of DNA polymerase II-mediated frameshift mutagenesis

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***Escherichia coli* possesses three SOS-inducible DNA polymerases (Pol II, IV, and V) that were recently found to participate in translesion synthesis and mutagenesis. Involvement of these polymerases appears to depend on the nature of the lesion and its local sequence context, as illustrated by the bypass of a single *N*-2-acetylaminofluorene adduct within the *NarI* mutation hot spot. Indeed, error-free bypass requires Pol V (*umuDC*), whereas mutagenic (−2 frameshift) bypass depends on Pol II (*polB*). In this paper, we show that purified DNA Pol II is able *in vitro* to generate the −2 frameshift bypass product observed *in vivo* at the *NarI* sites. Although the Δ*polB* strain is completely defective in this mutation pathway, introduction of the *polB* gene on a low copy number plasmid restores the −2 frameshift pathway. In fact, modification of the relative copy number of *polB* versus *umuDC* genes results in a corresponding modification in the use of the frameshift versus error-free translesion pathways, suggesting a direct competition between Pol II and V for the bypass of the same lesion. Whether such a polymerase competition model for translesion synthesis will prove to be generally applicable remains to be confirmed.**

NarI mutation hot spot | translesion synthesis | slippage mutagenesis | *N*-2-acetylaminofluorene | *umuDC* (Pol V)

Point mutations are formed during DNA replication either as genuine replication errors or as a consequence of the presence of damage in the parental DNA. By changing the chemical structure of the bases, damaging agents are often effective blocks to the progression of replicative DNA polymerases. It has now become clear that these blocks are overcome by specialized enzymes (translesional DNA polymerases) that can read through damaged bases in a process known as translesion synthesis (TLS) (1–5). Because of the presence of the lesion, this process is inevitably less accurate than normal replication and will thus trigger increased mutation rates in the newly synthesized strand opposite the damaged base.

Recently it was shown that in *Escherichia coli*, all three SOS-inducible DNA polymerases, Pol II (*polB*), Pol IV (*dinB*), and Pol V (*umuDC*), can be involved in TLS, depending on the nature of the DNA damage and its sequence context (6). We have identified an intriguing situation where the bypass of a given lesion, *N*-2-acetylaminofluorene (AAF), located within a frameshift mutation hot spot, the *NarI* site, is mediated by two genetically distinct pathways. Indeed, in that sequence context, the bypass of an AAF guanine adduct requires Pol V for nonslipped elongation, yielding error-free TLS but Pol II for slipped elongation, thus producing −2 frameshift TLS (6). The nonslipped TLS pathway obeys the rules previously described for base substitution mutagenesis induced by UV light or abasic sites, i.e., Pol V and RecA* dependence (for a recent review, see ref. 7). In contrast, the involvement of DNA Pol II in the slipped −2 frameshift pathway is more intriguing. Although a series of phenotypes related to DNA repair have been described in *polB* strains (8–12), only one study has pointed to the potential role of the *polB* gene product in the bypass of abasic sites *in vivo* (13).

In this paper, we show that purified DNA Pol II is able *in vitro* to generate the −2 frameshift bypass product observed *in vivo* at the *NarI* sites. Moreover, we analyze *in vivo* the relative utilization of the Pol II versus Pol V pathways on introduction into the Δ*polB* strain of the *polB* gene on a low copy number plasmid and show that the two polymerases compete for elongation of the same replication intermediate.

Experimental Procedures

Construction of *polB* (exo+ and exo−) Expression Vectors. The coding sequence of the *polB* gene was amplified by PCR from genomic DNA isolated from strain MG1655. The natural but ''inefficient'' GTG translation initiation codon was changed to ATG during PCR amplification. The corresponding 2.4-kb PCR product was first purified after agarose gel electrophoresis and inserted into the pCAL-n-FLAG expression vector (Stratagene). The selected recombinant plasmids pCAL-n-FLAG-*polB* were digested and partially sequenced to confirm the identity of the 2.4-kb insert. An exo− version of the *polB* gene containing two substitutions (D155A and E157A) in the Exo I motif was engineered by using standard oligonucleotide site-directed mutagenesis. The expected mutations were confirmed by sequencing.

Purification of DNA Polymerase II. *E. coli* strain BL21(DE3) transformed with pCAL-n-FLAG-*polB* were grown at 37°C in 500 ml of LB containing 100 μg/ml of ampicillin to an OD_{600} of 0.6. Induction of *polB* expression was started by adding isopropyl β-**D**-thiogalactoside to a final concentration of 1 mM. Expression was carried out overnight at 17°C to prevent inclusion bodies. Cells were harvested by centrifugation, the pellet was washed with buffer A (50 mM Tris·HCl, pH 8.0/300 mM NaCl/10 mM β-mercaptoethanol/1 mM magnesium acetate/1 mM imidazole/2 mM $CaCl_2$), resuspended in 10 ml of the same buffer, and frozen at −80°C. The frozen cell suspension was thawed and supplemented with 1 mg of chicken egg lysozyme (Boehringer Mannheim) and protease inhibitors (Complete EDTA free, Roche Biochemicals). Cells were disrupted by sonication, and the insoluble debris were removed by centrifugation at 16,000 × *g* at 4°C for 30 min. The clarified supernatant (10 mg/ml of total protein) was applied to a Calmodulin affinity resin. After loading, the column was washed with 10 vol of buffer A followed by 5 vol of buffer A supplemented with 1 M NaCl final. The CBP-tagged Pol II was eluted with 5 vol of buffer B (50 mM Tris·HCl, pH 8.0/300 mM NaCl/10 mM β-mercaptoethanol/2 mM EGTA) and 5 vol of buffer B supplemented with 1 M NaCl

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Abbreviations: TLS, translesion synthesis; AAF, *N*-2-acetylaminofluorene; SSB, single-strand-binding protein; LT, lesion terminus.

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final. The eluted fractions were pooled and dialyzed against buffer C (50 mM Tris·HCl, pH 8.0/50 mM NaCl/2 mM $CaCl_2$/0.1% Tween-20). Protein concentration was determined by the Bradford assay. Native DNA Pol II was obtained by proteolytic cleavage of 200 μg of CBP-tagged Pol II with 2 units of enterokinase (Stratagene) in buffer C at 4°C overnight. DNA Pol II was stored in buffer D (50 mM Tris·HCl, pH 8.0/200 mM NaCl/5 mM DTT/50% glycerol) at −80°C. The Pol II exo− protein was expressed and purified in a similar way. Both purified DNA Pol II exo− and exo+ preparations appear as a single polypeptide of ≈90 kDa (purity was estimated to be ≥95%) on 8% SDS/PAGE after Coomassie blue staining.

Determination of DNA Pol II Specific Activity. The specific activity of native Pol II was measured by using a classical incorporation assay with a mixture of all four dNTPs at 100 μM final containing α-^{32}P-dCTP (30 Ci/mmol, Amersham Pharmacia). Reactions contained 0.1 pmol of single-stranded pUC118 DNA (≈10 ng) annealed to a 24-mer oligonucleotide primer/20 mM Tris·HCl (pH 8.0)/10 mM $MgCl_2$/5 mM DTT/0.1 mg/ml BSA/5% glycerol/0.01 pmols of purified DNA Pol II. By definition, 1 unit of enzyme catalyzes the incorporation of 1 pmol of α-^{32}P-dCTP into insoluble material in 1 min at 37°C.The specific activity of our preparation of native wild-type Pol II was found to be 1.5×10^4 units/mg, in good agreement with the previously published value (14). The specific activity of Pol II exo− was 3-fold lower than that of the wild-type Pol II, suggesting that the D155A and E157A substitutions cause a small reduction in enzyme activity (14). No 3′-5′ exonuclease activity was detected with Pol II exo−.

Oligonucleotide Primer/Templates. Oligonucleotides containing a single T(6–4)T photoproduct or a single G-AAF adduct within the 3G or the *NarI* sequence context have been described previously (15–17). To serve as templates in primer extension reactions, these oligonucleotides were extended by ligation on their 3′-ends to a common 55-mer oligonucleotide by using complementary scaffold oligonucleotides generating the series of "70-mer" templates. Similarly a series of "90-mer" templates was constructed by simultaneous ligation of a 20-mer and a 55-mer onto the 5′ and 3′ ends of the lesion containing oligonucleotide. Annealing of the scaffold, ligation, and subsequent purification by PAGE of the ligation product were done as described previously (18–20). After purification, these 70- or 90-mer template oligonucleotides are annealed to a 24- or 20-mer primer that is complementary to the 55-mer oligonucleotide common to all templates. Sequences of the oligonucleotides templates (70 mers): NarAAF template: 5′TACACCGGCGCCACAGACTAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGG3′, 3G template: 5′AATTCAGTCATACCCGGGACATCGACTAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGG3′ and T(6–4)T sequence: 5′GCAAGTTAACACGGACTAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGG3′ (target site is underlined). Twenty mer used in the construction of the 90 mers: 5′CCATGATTACGAATTCAGTC3′.

Primer Extension Reactions. All reactions were conducted at 30°C in the following buffer: 20 mM Tris·HCl (pH 8.0)/10 mM $MgCl_2$/5 mM DTT/1 mM ATP/0.1 mg/ml BSA/dATP, dTTP, dGTP, dCTP each at 200 μM final. Amounts of primer-template, single-strand-binding protein (SSB), and polymerase are specified in the legend of the figures. Klenow fragment (exo−) was purchased from New England Biolabs. Reactions were terminated by the addition of 4 vol of formamide containing 20 mM EDTA and bromophenol blue. The reaction mixtures were heat denatured, electrophoresed on 12 or 15% denaturing (8 M urea) polyacrylamide gels, and visualized by using a PhosphorImager 445SI (Molecular Dynamics). The identity of given replication products was established after recovery of the product from the gel and sequencing by means of the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (Amersham Pharmacia).

Bacterial Strains and Plasmids. *E. coli* strain MGZ and its Δ*polB* derivative have been previously described (6). The *polB* gene expressed from its natural promoter/operator region has been cloned into the low copy number vector pWKS130 derived from pSC101 (21).

Determination of Error-Free and Mutagenic TLS *in Vivo*. The relative proportion of error-free and −2 frameshift TLS events were determined *in vivo* as described previously by using a plasmid with a single AAF adduct located within a *NarI* sequence 5′-GGCG^{AAF}CC-3′ in the N-terminal part of the *lacZ′* gene of a pUC-derived plasmid (22, 23). This construction, introduced into SOS-induced bacteria by electroporation, confers a $LacZ^-$ phenotype that can be reverted to $LacZ^+$ by a −2 frameshift mutation (22, 23). SOS induction was achieved by prior UV irradiation at a dose of 50 J/m^2, as previously described (24). The fraction of −2 frameshift TLS events is determined as the fraction of blue colonies among all transformants on indicator plates containing carbenicillin, 5-bromo-4-chloro-3-indolyl β-**D**-galactoside, and isopropyl β-**D**-thiogalactoside. Colonies resulting from either error-free TLS or damage avoidance events form white colonies on these plates. Among the white colonies, the fraction of colonies resulting from error-free TLS is determined by using the colony hybridization assay developed previously (22, 24). The majority of colonies represent colonies where plasmid replication occurred via damage avoidance (22, 24, 25).

Results

For the sake of clarity, we will define a few terms to characterize the process of TLS. A TLS reaction may be viewed as comprising at least two steps: (*i*) an *insertion step*, during which a nucleotide is incorporated in the newly synthesized strand across from the lesion. This step generates a key replication intermediate that we refer to as the lesion terminus (LT) (26, 27); and (*ii*) one or several *extension step*(s) of the LT.

DNA Pol II Generates Frameshift Products During Bypass of an AAF Adduct Located Within the NarI Site. All TLS reactions are performed by using synthetic primer/template oligonucleotides. Primers are designated as L*n*, *n* being the number of nucleotides between the 3′-end of the primer and the lesion site L. For example, L0 and L-6 refer to primers whose 3′ end is located across and six nucleotides upstream from the lesion site, respectively.

A template oligonucleotide containing a single AAF adduct located at G_3 within the *NarI* site ($G_1G_2C\mathbf{G}_3^{AAF}CC$) was annealed to primer L-6 and incubated with DNA Pol II. Primer elongation products are visualized on sequencing gels. In addition to a strong stop at position L-1, both partial and complete bypass products were observed in the presence of DNA Pol II (Fig. 1*A*). Compared with the lesion-free control template, no full length replication product is obtained with the AAF-containing template. Instead, two bypass products of similar intensities, respectively one and two nucleotides shorter than the full length products, are observed (Fig. 1*A*). The sequence of the (−1) and (−2) bypass products was determined. The (−2) bypass product is the expected *NarI* frameshift mutation resulting from C incorporation across G-AAF followed by a two-nucleotide slippage reaction (i.e., GGC$\mathbf{G}^{AAF}$CC→GGCC), whereas the (−1) product is "*compatible*" with misincorporation of G across G-AAF, followed by a one-nucleotide slippage reaction (GGC$\mathbf{G}^{AAF}$CC→GGGCC), as described in the scheme below (Scheme 1). The −1 product may also be produced if Pol II

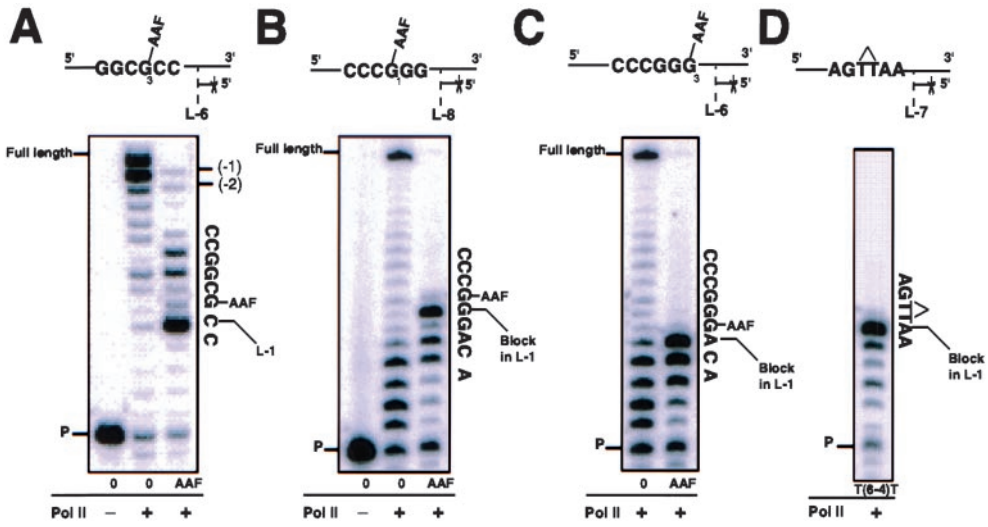


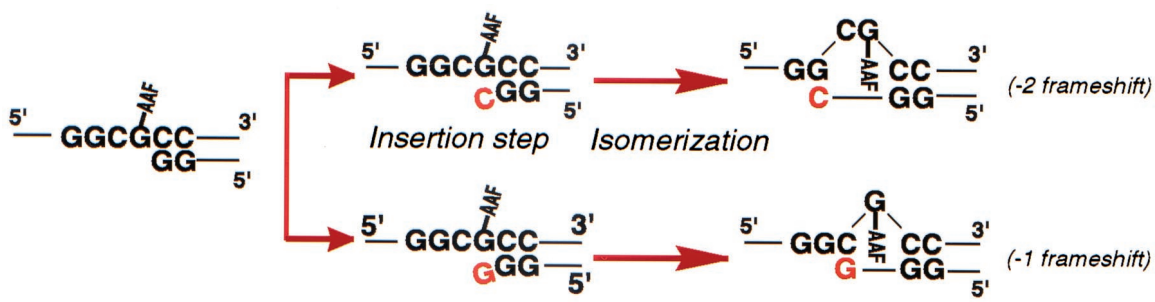
Fig. 1. Primer elongation experiments by Pol II on AAF- and UV-damaged DNA templates. The substrates used in this set of experiments consisted of a 24-mer primer (5′-^{32}P labeled) annealed to 70-mer templates. Templates contained either no lesion, single AAF in various sequence contexts (as shown), or a single T(6–4)T photoproduct. The 3′-end of the primer is located several nucleotides upstream from the lesion, as indicated. All reactions were performed under the standard reaction conditions (see *Material and Methods*) by using 1 nM primer/template coated with SSB (1 molecule of SSB per nucleotide) and 10 nM of Pol II (0.03 units). Reactions were performed at 30°C for 5 min, quenched with 20 mM EDTA, and analyzed by 12% denaturing polyacrylamide gels (8 M urea). Presence or absence of the lesion is symbolized by AAF or 0, respectively, whereas + and − indicate, respectively, the presence or absence of a given polymerase. Substrate structure is shown on top. (*A*) AAF adduct within the *NarI* (GGCG^{AAF}CC) sequence. (*B* and *C*) AAF adduct within sequence $G_1G_2G_3$, on G_1 or G_3. (*D*) Template with a T(6–4)T photoproduct.

"skips" over the G-AAF adduct via a dNTP stabilized looped-out structure (28, 29).

TLS experiments described above, involving primer L-6, are generally referred to as "running start" reactions. By using primer L-1, i.e., "standing start" conditions, a similar bypass pattern involving the formation of both (−1) and (−2) TLS products is also observed (data not shown). In contrast, when Pol II is used for the extension reaction, with primer L0, only the (−2) bypass product is formed (see below).

Similar TLS reactions were conducted by using a single G-AAF adduct located within a different sequence context, namely within sequence 5′-CG$_1$G$_2$G$_3$A-3′, a sequence previously identified as a −1 frameshift mutation hot spot (16, 30, 31). The AAF adduct was located either at G_1 or G_3 positions. With both substrates, elongation of the corresponding primer proceeds to the position preceding the lesion site (L-1) forming a strong block (Fig. 1 *B* and *C*). No incorporation across or beyond the lesion site is seen (Fig. 1 *B* and *C*). These results are in agreement with the genetic data showing that Pol II is involved neither in error-free nor mutagenic bypass of AAF adducts within this sequence context (6). Indeed, Pol V (*umuDC*) mediates these bypass reactions (6, 24). Similarly, replication of an oligonucleotide containing a single T(6–4)T photoproduct shows that Pol II elongates the primer to the position preceding the 3′-T of the photoproduct, without incorporation across or beyond the lesion site (Fig. 1*D*). These results are also in agreement with the *in vivo* data, as Pol V was shown to be required in the bypass of T(6–4)T lesions (6, 15).

Characterization of the Frameshift Replication Intermediate Formed During the Bypass of an AAF Adduct by DNA Pol II. Extension experiments were carried out with a primer/template substrate that mimics the LT. The 3′-end of the primer was located across from the lesion site (primer L0) with a C residue across from the G-AAF adduct, as it was shown both *in vivo* and *in vitro* that most of the time G-AAF adducts "correctly" direct the insertion of cytosine (26, 32). Pol II is able to extend this intermediate generating exclusively bypass product two nucleotides shorter than the one obtained with the lesion-free control template (Fig. 2). Sequencing of the (−2) bypass product reveals that TLS occurred with the loss of a CpG dinucleotide (5′-GGCGCC→5′-GGCC), strongly suggesting the elongation of a two-nucleotide slippage intermediate (Fig. 5). The capacity of wild-type Pol II to specifically extend such an unusual primer terminus is surprising in view of its robust 3′-5′ exonuclease activity (see discussion below) (14). In fact, an exonuclease-deficient mutant of Pol II (D155A and E157A in the ExoI motif) yields a similar amount of (−2) bypass product (Fig. 2). We have no explanation for the presence of a pause site at L + 1 for both



Scheme 1.

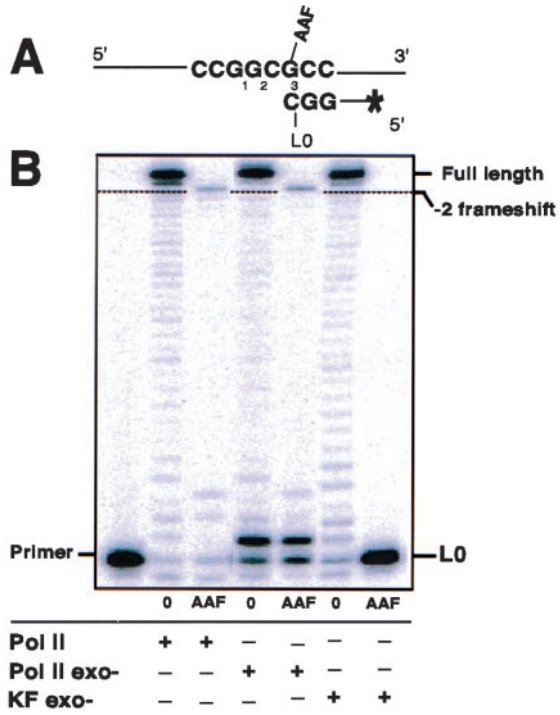


Fig. 2. LT extension reactions. (*A*) The primer/template (20 mer/90 mer) used in this set of experiments mimics the LT at the *NarI* site, as the 3′-end of the primer is located across from the lesion site. (*B*) Reaction and analysis conditions as described in Fig. 1. Amount of polymerases, Klenow fragment exo− and Pol II exo+ and Pol II exo− were adjusted to provide the same efficiency of elongation on the nondamaged template for direct comparison 0.03 units of Pol II, 0.09 units of Pol II exo−, and 0.05 units of Klenow fragment exo−.

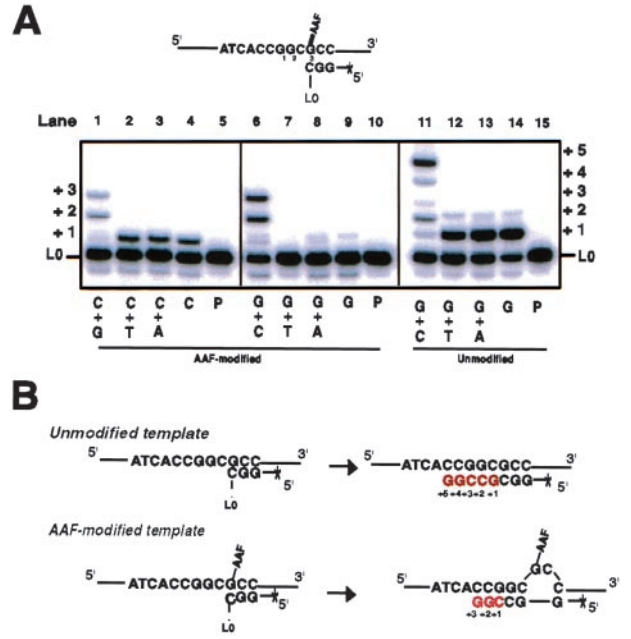


Fig. 3. Replication intermediates formed during the extension of the *NarI* LT by Pol II. (*A*) Structure of the primer-template sequence is shown on top. Pol II (2 nM) and 1 nM SSB-coated primer/template (20 mer/70 mer) were incubated and analyzed under standard conditions in the presence of one or two dNTPs (100 μM each), as indicated. (*B*) Extension intermediates on unmodified and AAF-modified *NarI* templates. Structure of the potential replication intermediates taking into account the preferential incorporation at the +1 position of C and G with the AAF-modified and unmodified templates, respectively.

modified and unmodified substrates with the Pol II exo− enzyme. The exo− Klenow fragment is unable to extend this replication intermediate.

For further insight into the mechanism of the −2 bypass reaction, we conducted extension reactions by using only one or a combination of two dNTPs. Extension of primer L0 annealed to the lesion-free control template gave the expected results (Fig. 3). With dGTP or a combination of dGTP and either dTTP or dATP only one nucleotide, (G) is incorporated (Fig. 3*A*, lanes 12–14). With a mixture of dGTP and dCTP, a five-nucleotide extension of the primer is observed, as expected from the template sequence (Fig. 3*A*, lane 11). With the AAF-containing template, no extension of the primer is observed when incubated with dGTP or a combination of dGTP and either dTTP or dATP (Fig. 3, lanes 7–9). In contrast, when incubated with dCTP or a combination of dCTP and dTTP or dATP, a one-nucleotide extension reaction is observed (Fig. 3*A*, lanes 2–4). With dGTP and dCTP, the primer is elongated by three nucleotides (Fig. 3*A*, lanes 1 and 6). The specificity of the observed extension reaction is compatible with the formation of a slipped primer/template intermediate, as shown in Fig. 3*B*. The 5′-GpC extremity of the primer has slipped onto the complementary 5′-GpC sequence that resides next to the G-AAF adduct on the template strand. As a result, the 5′-G^{AAF}pC dinucleotide in the template strand remains unpaired. We have previously shown that the G-AAF adduct stabilizes such a slipped structure by virtue of stacking interactions between the aromatic AAF moiety with the adjacent bases (33–35). We suggest that the LT can adopt two conformations. In the nonslipped conformation, the 3′-end of the primer is likely to be "breathing" because of the presence of the G-AAF adduct in the template strand. Indeed, AAF adducts have been shown to induce local opening of double-stranded DNA (described as the Insertion Denaturation Model) (36, 37). In contrast, in its slipped conformation, the 3′-end of the primer forms two correct GC base pairs at its extremity. This particular structure of the primer/template appears to be efficiently extended by Pol II, thus generating −2 frameshift mutations. The stability of the primer terminus that is brought about by the two terminal GC base pairs in the slipped intermediate may be enough to prevent the proofreading reaction.

Expression of Pol II from a Low Copy Number Plasmid Complements the Defect in NarI Mutagenesis of a ΔpolB Strain. To check whether the expression of Pol II is able to restore *NarI* frameshift mutagenesis in a Δ*polB* strain, we cloned the wild-type *polB*$^+$ gene expressed from its natural SOS-controlled promoter into a low copy number plasmid (pWKS130) derived from pSC101 (four to eight copies/cell) (21). The resulting plasmid pWKS-*polB*$^+$, or the control vector pWKS130, was introduced into a Δ*polB* strain. TLS was analyzed in the resulting strains under SOS-induced conditions by using a vector with a single AAF adduct located within the *NarI* site, as described previously (6, 22). This vector contains a small sequence heterology in the vicinity of the AAF lesion that serves as a genetic strand marker, allowing the quantitative determination of both error-free and mutagenic TLS under *in vivo* replication conditions (22, 24). As expected from previous results, when comparing the wild-type strain to the Δ*polB* strain containing the control vector, frameshift TLS was completely abolished, whereas error-free TLS remained mostly unaffected (Fig. 4) (6). On introduction of the *polB*$^+$ plasmid in the Δ*polB* strain, mutagenic TLS increases from ≈0.1 to ≈40%, demonstrating that Pol II expression is sufficient to restore *NarI* frameshift mutagenesis (Fig. 4). A drastic change in the TLS pattern is observed when comparing the wild-type strain containing one copy of the *polB* gene to the strain containing several copies of *polB*. Indeed, a strong increase in slipped frameshift TLS from ≈10 to

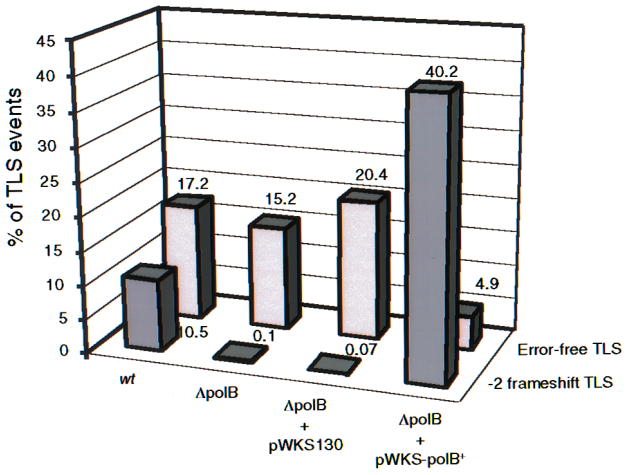


Fig. 4. Extent of error-free and −2 frameshift TLS events *in vivo*. Error-free and −2 frameshift TLS events measured *in vivo* in different SOS-induced strains: wt, wild type strain MGZ; Δ*polB*, strain MGZΔ*polB*; Δ*polB* + pWKS130, strain MGZΔ*polB* transformed with pWKS130. Δ*polB* + pWKS-*polB*$^{+}$: strain MGZΔ*polB* transformed with the corresponding *polB*+ gene-expressing plasmid.

≈40% with a concomitant decrease in error-free TLS from ≈20 to ≈5% is observed (Fig. 4). The potential biological meaning of this observation will be discussed below.

Discussion

To date, the genetics and biochemistry of lesion bypass have mostly been inferred from work with UV photoproducts and abasic sites as model lesions. Bypass of these lesions requires both the *umuDC* (Pol V) and *recA* gene products (38). The bypass in *E. coli* of other replication-blocking lesions, such as adducts formed by polycyclic hydrocarbons and aromatic amides, has recently been shown to potentially involve all three SOS-inducible DNA polymerases (Pol II, IV, and V) (6).

We have identified a situation where the bypass of a given lesion, AAF, located within a frameshift mutation hot spot, the *NarI* site, is mediated by two distinct pathways: Pol V and II are required for nonslipped (error-free) and slipped −2 frameshift TLS, respectively (6).

Biochemical Model of TLS at the NarI Site. Within the frame of the two-step model of TLS, both the *insertion* and *extension* step(s) present distinct challenges to polymerases. The insertion step entails the addition onto a nondistorted primer of a nucleotide across from a damaged template base, whereas the reverse situation occurs for the extension step(s), i.e., addition onto a distorted primer terminus of a nucleotide across from a non-damaged template position. One or a combination of several specialized DNA polymerases may perform the insertion and extension steps. Our data allow us to propose a model specifying the polymerases involved *in vivo* in the insertion and extension steps during the bypass of AAF within the *NarI* site.

Insertion Step. In the present paper, we show that when Pol II is used to extend the LT, it is indeed able to generate exclusively the −2 TLS products that are observed *in vivo*. In contrast, when Pol II is used in the complete TLS reaction, involving both insertion and extension steps, both −1 and −2 TLS products are observed. That −1 frameshift mutations are not found at *NarI* sites *in vivo* (29, 39) suggests that the reaction leading to the −1 TLS product *in vitro* is not biologically relevant. Therefore, most likely, *in vivo*, Pol II is involved not in the insertion step but only in the extension step(s). The question remains as to which DNA polymerase(s) mediate(s) the insertion reaction *in vivo*? We have previously shown that −2 frameshift TLS is fully proficient in a Δ*dinB* strains but is reduced by about 30% in a Δ*umuDC* strain, suggesting thus Pol V but not

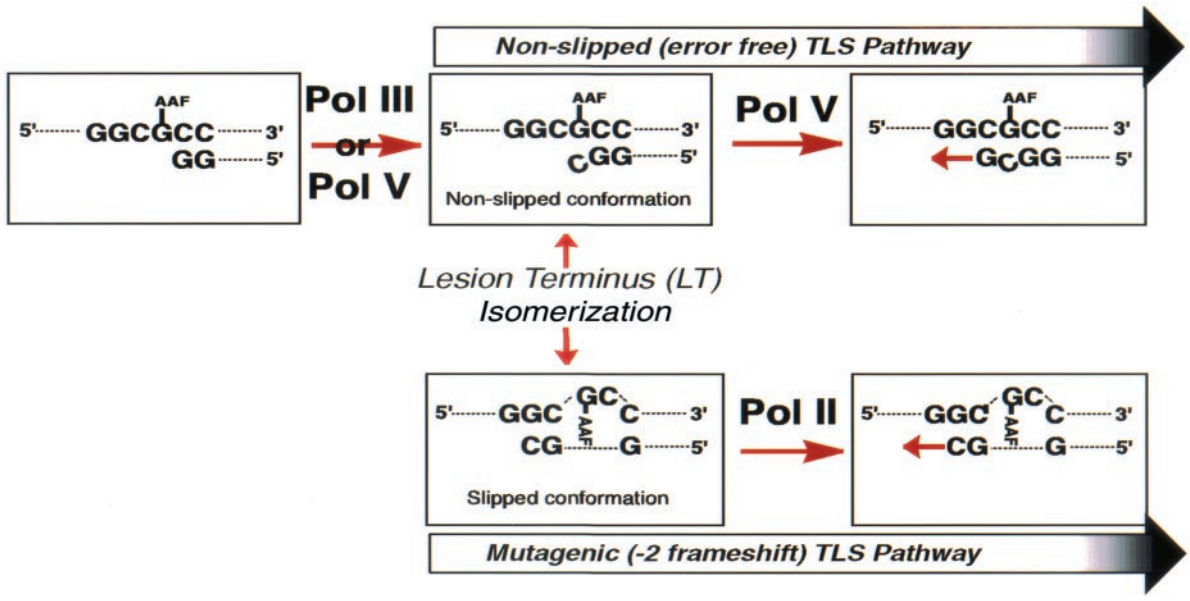


Fig. 5. Model for TLS pathways at an AAF adduct located within the *NarI* site. Two distinct pathways for the bypass of a single AAF adduct located within the *NarI* site can be defined. The nonslipped pathway yielding error-free TLS requires the SOS-inducible DNA Pol V and RecA* similarly to UV-induced base substitution mutagenesis pathway. In contrast, the slipped pathway that generates −2 frameshift mutations requires another SOS-inducible DNA polymerase, namely DNA Pol II. Both nonslipped and slipped pathways are of similar importance in a wild-type strain representing 17 and 10% of the replication events, respectively. *In vivo* and *in vitro* studies allow us to describe the individual steps involved in both pathways. Formation of the LT by insertion of C opposite the G-AAF adduct appears to be feasible by either Pol III or Pol V. This replication intermediate, common to both pathways, can adopt two conformations referred to as nonslipped and slipped conformations. The nonslipped conformation exhibits a distorted 3′-end of the primer, whereas in its slipped conformation, the primer forms two correct GC base pairs at its extremity. *In vivo* analysis of TLS reveals a competition between Pol V and II for the elongation of the nonslipped and slipped lesion termini, respectively.

Pol IV may act at the insertion step *in vivo* (6, 40). However, as the effect of Δ*umuDC* is only partial (30% reduction), we suggest that, in addition to Pol V, Pol III itself can perform the insertion step.

Extension Step. Depending on its conformation, the same LT can be extended by two different DNA polymerases, generating distinct bypass products (Fig. 5). In the nonslipped conformation, Pol V appears to be able to extend the LT despite the fraying of the 3′-end extremity of the primer yielding an error-free bypass product. This pathway appears to be similar to UV photoproduct or abasic site TLS pathways. In contrast, in its slipped conformation, when the primer template terminus exhibits two correct GC base pairs, TLS is mediated efficiently by Pol II yielding −2 frameshift mutations. It should be stressed that in wild-type *E. coli*, both pathways are of similar relative importance (Fig. 4). It is not yet clear whether the slipped intermediate of the LT exists in solution or is formed as a consequence of Pol II binding.

TLS: A Competition Between DNA Polymerases? When the replicative DNA polymerase (Pol III) encounters a replication-blocking lesion, it is likely that it detaches from the primer terminus. However, the processivity clamp remains on the DNA and may act as a common plate-form for the recruitment of the translesional DNA polymerases. Indeed, the polymerase activity of Pol II (41), Pol IV (42), and Pol V (43) are known to be stimulated by the processivity clamp. Interestingly, an increase in the level of expression of Pol II relative to that of Pol V *in vivo* results in a corresponding increase in the amounts of slipped bypass with a concomitant decrease in nonslipped bypass (Fig. 4). In fact, Pol II and V appear to compete for the extension of this LT. In the wild-type strain, Pol II and V pathways represent about 40 and 60% of the TLS events, respectively. In the Δ*polB* strain, the Pol II pathway represents less than 1% of all TLS events. On introduction of the *polB* plasmid, the Pol II pathway amounts to 90% of all TLS events, whereas the Pol V pathway represents about 10%. Modulation of the extent of total TLS in the different strains reflects the competition between TLS and damage avoidance events.

Even if the situation described here is restricted to this particular combination of "local sequence/specific adduct," it nevertheless tells us that the "classical" TLS pathway involving DNA Pol V can be challenged by another DNA polymerase, namely by DNA Pol II. Current models of TLS propose that Pol V is positioned at the lesion site by means of a RecA/single-stranded DNA filament (44, 45). Our data suggest that the LT is available for Pol II extension (in its slipped conformation) in a reaction that competes with the Pol V/RecA* pathway. Both prokaryotic and eukaryotic cells possess a pool of DNA polymerases, with relaxed specificities to deal with the large variety of existing DNA lesions. The specific role of the various DNA polymerases is not yet clear. Some polymerases may be specialized in the insertion step, whereas others are specialized in the subsequent extension steps. Moreover, as the gaps that are generated *in vivo* opposite replication blocking lesions are up to 1,000 nucleotides long (46), the question also remains as to which DNA polymerase(s) performs the bulk of the gap-filling reaction. Whether a TLS model based on the competition among DNA polymerases (as suggested by the present work) will prove to be generally applicable remains to be further documented.

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